

CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A recombinant vector for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA, comprising from 5' to 3':
 - a) an RNA polymerase II dependent promoter sequence derived from the U1 snRNA gene;
 - b) suitable restriction sites for cloning the sequence that transcribes a presiRNA or a pre-miRNA;
 - c) a sequence transcribing the pre-siRNA comprising: in position +1 an A or a G residue; a sequence from 21 to 23 nucleotides corresponding to a sense region of the mRNA transcribed by the gene to be silenced, that constitutes the first segment of the stem of the pre-siRNA; a sequence selected to form a pre-miRNA sequence that constitutes the loop region of the pre-siRNA; a sequence from 21 to 23 nucleotides corresponding to the antisense region of the mRNA transcribed by the gene to be silenced that constitutes the second segment of the stem of the pre-siRNA; wherein the residues at the 3' end protrude in such a way that the following structure is obtained:

5'-YA/G----sense sequence-----
3'-XUU/C-antisense sequence-----
loop

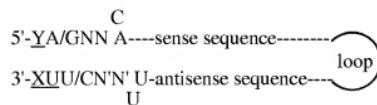
or alternatively a sequence transcribing the pre-miRNA, wherein X and Y is [[are]] optionally present, and if present X and Y if Y is present are independently one or more nucleotides that are complementary, wherein the nucleotides at the 3' end are selected to result in asymmetry in siRNA strand selection into an interference complex; and

- d) termination sequences derived from the sequence at 3' of the gene for U1 snRNA which are necessary and sufficient for the correct formation of the 3' of the pre-siRNA or of the pre-miRNA, wherein the recombinant vector provides for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA.

2. (Original) The vector according to Claim 1, wherein the cloning site for the 5' of the sequence transcribing the pre-siRNA is BgI II.

3. (Currently Amended) The vector according to Claim 1, wherein the sequence transcribing the pre-siRNA further comprises at termini 5' and 3' such sequence that the transcribed pre-siRNA has the following structure A recombinant vector for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA, comprising from 5' to 3':

- a) an RNA polymerase II dependent promoter sequence derived from the U1 snRNA gene;
- b) suitable restriction sites for cloning the sequence that transcribes a presiRNA or a pre-miRNA;
- c) a sequence transcribing the pre-siRNA comprising: in positions +1 an A or a G residue; a sequence from 21 to 23 nucleotides corresponding to a sense region of the mRNA transcribed by the gene to be silenced, that constitutes the first segment of the stem of the pre-siRNA; a sequence selected to form a pre-miRNA sequence that constitutes the loop region of the pre-siRNA; a sequence selected from 21 to 23 nucleotides corresponding to the antisense region of the mRNA transcribed by the gene to be silenced that constitutes the second segment of the stem of the pre-siRNA; wherein the residues at the 3' end protrude in such a way that the following structure is obtained:

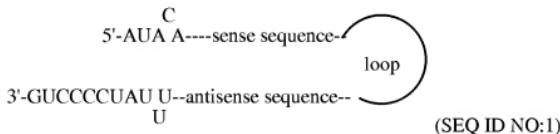


wherein N is A, U, G or C and N' is its complementary nucleotide, wherein Y is optionally present, wherein X and Y if Y is present are independently one or more nucleotides, wherein the nucleotides at the 3' end are selected to result in asymmetry in siRNA strand selection into an interference complex; and

- d) termination sequences derived from the sequence at 3' of the gene for U1 snRNA which are necessary and sufficient for the correct formation of the 3' of the pre-siRNA or of the pre-miRNA, wherein the recombinant vector provides for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA.

4. (Currently Amended) The vector according to Claim 3, wherein the sequence transcribing the pre-siRNA comprises at the 5' and 3' termini such sequences that the transcribed pre-siRNA has the following structure A recombinant vector for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA, comprising from 5' to 3':

- a) an RNA polymerase II dependent promoter sequence derived from the U1 snRNA gene;
- b) suitable restriction sites for cloning the sequence that transcribes a presiRNA or a pre-miRNA;
- c) a sequence transcribing the pre-siRNA comprising: in position +1 an A or a G residue; a sequence from 21 to 23 nucleotides corresponding to a sense region of the mRNA transcribed by the gene to be silenced, that constitutes the first segment of the stem of the pre-siRNA; a sequence selected to form a pre-miRNA sequence that constitutes the loop region of the pre-siRNA; a sequence from 21 to 23 nucleotides corresponding to the antisense region of the mRNA transcribed by the gene to be silenced that constitutes the second segment of the stem of the pre-siRNA; wherein the residues at the 3' end protrude in such a way that the following structure is obtained:



; and

- d) termination sequences derived from the sequence at 3' of the gene for U1 snRNA which are necessary and sufficient for the correct formation of the 3' of the pre-siRNA or of the pre-miRNA, wherein the recombinant vector provides for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA.

5. (Previously Presented) The vector according to claim 4 wherein the termination sequences derived from the sequence at 3' of the gene for U1 snRNA are as follows:

CCCTTG/ACTTTCTGGAGTTCAAAAGTAGAC (SEQ ID NO:18).

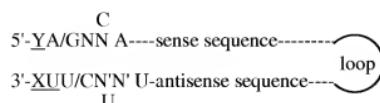
6. (Previously Presented) The vector according any of claims 1 to 5 further comprising suitable sequences to make inducible the RNA pol II promoter.

7. (Currently Amended) A composition for gene therapy comprising the vector according to claim 1,3, 4 or 10.

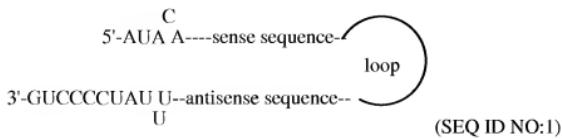
8-9. (Canceled).

10. (Currently Amended) A recombinant vector for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA, comprising from 5' to 3':

- a) an RNA polymerase II dependent promoter sequence derived from the U1 snRNA gene;
- b) suitable restriction sites for cloning the sequence that transcribes a presiRNA or a pre-miRNA;
- c) a sequence transcribing the pre-siRNA comprising: in position +1 an A or a G residue; a sequence from 21 to 23 nucleotides corresponding to a sense region of the mRNA transcribed by the gene to be silenced, that constitutes the first segment of the stem of the pre-siRNA; a sequence selected to form a pre-miRNA sequence that constitutes the loop region of the pre-siRNA; a sequence from 21 to 23 nucleotides corresponding to the antisense region of the mRNA transcribed by the gene to be silenced that constitutes the second segment of the stem of the pre-siRNA; wherein one of the following structures is obtained:



wherein where N is A, U, G or C and N' is its complementary nucleotide, wherein Y is optionally present, wherein X and Y if Y is present are independently one or more nucleotides, wherein the nucleotides at the 3' end are selected to result in asymmetry in siRNA strand selection into an interference complex, or



or alternatively a sequence transcribing the pre-miRNA; and

d) termination sequences derived from the sequence at 3' of the gene for U1 snRNA which are necessary and sufficient for the correct formation of the 3' of the pre-siRNA or of the pre-miRNA.